CERTAIN MEANS BY WHICH STARFISH EGGS NATURALLY RESISTANT TO FERTILIZATION MAY BE RENDERED NORMAL AND THE PHYSIOLOGICAL CONDITIONS OF THIS ACTION.

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Last summer at Woods Hole, while investigating the influence of ether and other anæsthetics in protecting the unfertilized eggs of starfish against the cytolytic action of pure isotonic sodium chloride solution,[1] I observed that toward the end of the breeding season the eggs often proved abnormally resistant to this solution, withstanding in some instances exposure of more than three hours to 0.55*m* NaCl without losing the power of development on fertilization; in normal eggs this solution typically causes complete cytolysis and coagulation of the protoplasm in two hours or less. Other characteristic abnormalities of behavior were found to be associated with this abnormal resistance to salt solutions. Usually a large proportion of such eggs failed to undergo maturation in sea-water, and of those which matured only a small proportion developed to a free swimming stage on fertilization, and the resulting larvæ were largely abnormal. Also the mature eggs, if left unfertilized in sea-water, frequently failed to die and break down within the usual time, but remained clear and apparently normal in appearance for an unusually prolonged period. It is well known that unfertilized mature starfish eggs undergo spontaneously a characteristic cytolytic alteration, accompanied by a darkening or coagulation of the protoplasm, which is typically complete within twelve to fifteen hours after deposition;[2] in contrast to this behavior a considerable proportion of the eggs under consideration often remained clear and uncoagulated in sea-water for twenty-four and in some cases for forty-eight hours. These several peculiarities, (1) failure of

[1] *American Journal of Physiology*, 1912, Vol. 30, p. 1.

[2] Cf. J. Loeb, *Archiv für die gesammte Physiologie*, 1902, Vol. 93, p. 59.

maturation in a large proportion of eggs, (2) failure of those eggs which did maturate to develop after fertilization, (3) marked delay in the typical post-maturational cytolysis of unfertilized eggs, and (4) unusual resistance to the cytolytic action of salt solutions were found in more or less constant association with one another in numerous lots of eggs. All of these peculiarities are evidence of a certain inertia or resistance to change in the egg-system; such eggs fail to react, or react slowly and imperfectly to conditions which call forth a definite and regular response in normal eggs. The fact of their concurrence, as well as their general nature, suggests that some single structural or metabolic abnormality, whose general effect is to lower reaction-velocities in the egg-system, is responsible for all of these peculiarities of behavior.

The further incidental observation was made in several instances that such eggs after treatment for two or three hours with sodium chloride solution containing a little ether or chloral hydrate recovered to a considerable degree the power of developing to a normal larval stage on fertilization; *i. e.*, eggs so treated yielded more numerous and more active larvæ than the untreated eggs of the same lot, fertilized at the same time. Pure sodium chloride solution showed a similar though less marked action. The surprising result thus appeared that treatment with solutions which are markedly injurious to normal eggs may bring a certain proportion of these abnormal or "over-ripe" eggs into a condition—as regards power of development on fertilization—closely approaching the normal.

The following record will illustrate the above-described conditions in detail.

June 29, 1911. The eggs were removed from a considerable number of starfish at 10.00 A.M. A large proportion of these mixed eggs remained permanently immature in sea-water (with intact germinal vesicles), but about half underwent apparently normal maturation. On fertilization (at 3:30 P.M.) very few of the eggs—a fraction of one per cent.—formed blastulæ, most died in early cleavage stages, but many failed to cleave or even to form fertilization-membranes. The unfertilized mature eggs after twenty-four hours in sea-water remained for the most part clear and translucent with no sign of coagulation; a good many, however, showed apparently normal coagulation, while others showed an intermediate condition. After forty-eight hours many unfertilized mature eggs were still uncoagulated.

About two hours after removal from the animals the unfertilized eggs were

placed in the following solutions: (1) pure 0.55*m* NaCl, and (2–10) 0.55*m* NaCl containing anæsthetics as follows: (2–4) ether, 0.6, 0.45, and 0.3 volumes per cent., (5–7) chloral hydrate, 0.6, 0.4 and 0.2 per cent., (8–9) chloroform, one sixth and one tenth saturated, and (10) ethyl alcohol, 5 volumes per cent. After three hours and fifteen minutes in these solutions the eggs were transferred to normal sea-water and washed free of the anæsthetics by two changes of sea-water; spermatozoa were then added. At the same time spermatozoa were added to the untreated eggs which had remained in sea-water. The results were as follows: of the control eggs, fertilized in sea-water without treatment, almost all died before reaching the blastula stage; only a few feeble blastulæ (a fraction of 1 per cent.) were found on careful search; many eggs failed to cleave or even to form membranes. The eggs exposed to pure 0.55*m* NaCl also formed few blastulæ, but these were relatively somewhat more numerous as well as more active than in the control; the eggs treated with 0.55*m* NaCl containing 0.45 and 0.3 vol. per cent. ether, especially the latter, showed a more decided improvement over the control, though the proportion of blastulæ was still small. The eggs from the other solutions showed no improvement.

A similar result was observed in a second series of experiments with eggs which showed similar peculiarities. Eggs treated for three hours with 0.55*m* NaCl containing 0.3 vol. per cent. ether gave about 5 per cent. of blastulæ, while of the control untreated eggs less than one per cent. reached this stage. Eggs similarly treated with solutions containing a higher proportion of ether (0.75, 0.6, and 0.45 vol. per cent.) showed no improvement over the control. In another series eggs exposed for 3 h. 45 m. to 0.55*m* NaCl containing 0.1 per cent. chloral hydrate gave considerably more blastulæ than the control eggs.

It is to be noted that the improvement in the developmental power of these abnormal or resistant eggs was produced only by the pure salt solution or by solutions with a *low* concentration of anæsthetic. The concentration of ether most favorable for retarding the cytolytic action of 0.55*m* NaCl is considerably higher—from 0.5 to 0.6 vol. per cent.[1] The present effect, however, is not due to a simple prevention of cytolysis; the improvement over eggs left in sea-water, none of which undergo cytolysis within the time of exposure, cannot thus be explained. The effect is different from a simply protective action; and since it seemed to be favored by weak solutions of ether, the experiment was tried of exposing a batch of similarly abnormal eggs to sea-water containing 0.3 vol. per cent. ether. After three hours the eggs were returned to normal sea-water and fertilized. Next day it was found that the great majority of mature eggs had formed

[1] Cf. R. S. Lillie, *loc. cit.*, p. 6.

active and vigorous blastulæ and gastrulæ, while of the untreated control eggs left in sea-water and fertilized at the same time as the others less than one per cent. formed larvæ and these were feeble and abnormal. It thus appeared that the abnormal condition which renders the egg incapable of responding fully to the fertilizing action of the spermatozoön might be removed by treatment with sea-water containing ether in certain concentrations, which are considerably lower than those required for typical anæsthetic or protective action.

What are the conditions of this effect? In a recent paper[1] I have presented evidence indicating that the protective and anæsthetic actions exerted by ether and other lipoid-solvents in certain concentrations are due primarily to a *modification of the plasma membranes* of the cells or irritable elements, of such a kind as to render these membranes more resistant toward agencies that under the usual conditions rapidly increase their permeability; cytolysis and stimulation, both of which depend on such increase of permeability, are hence checked or prevented. Decrease in the readiness with which the permeability is increased thus involves for an irritable tissue decreased irritability; this effect is produced by various salts, *e. g.*, of magnesium, and by ether and other lipoid-solvent anæsthetics in certain (not too high) concentrations. In lower concentrations it has been observed that ether and other lipoid-solvents frequently *heighten* irritability;[2] *i. e.*, expressed in terms of the membrane theory of stimulation, they increase the readiness with which the permeability—and hence the electrical polarization—of the plasma membrane undergoes change. It seems clear that for irritable tissues the state of the lipoids in the plasma membrane largely determines the readiness with which changes of permeability—and of the dependent electrical polarization—are induced by external agencies. Slight permeation of the lipoids with a lipoid-solvent like ether apparently often facilitates such changes and hence in-

[1] R. S. Lillie, *American Journal of Physiology*, 1912, Vol. 29, p. 372.

[2] For various instances of this effect cf. the references cited in my recent paper in *American Journal of Physiology*, 1912, Vol. 29, p. 374. The neuromuscular system of marine animals also shows it; *e. g.*, Bethe found that alcohol (0.5 per cent. in sea-water) decidedly increased the mechanical irritability of the isolated central portion of the medusa *Cotalorrhiza;* cf. "Allgemeine Anatomie und Physiologie des Nervensystems," Leipzig, 1903, p. 359.

creases irritability; the presence of more lipoid-solvent renders a change of permeability difficult,[1] hence the protective or anæsthetic action; while concentrated solutions of lipoid-solvents disrupt the membrane and produce cytolytic or irreversible alterations in the cells; hence such substances in higher concentrations are markedly toxic.

On the assumption that lipoid-solvents influence the plasma membranes of egg cells in essentially the same manner as those of irritable tissues, the above action of ether on abnormal egg cells becomes more readily intelligible; it falls, in fact, into the same essential category with the facts just cited. There is a close analogy between the stimulation of irritable tissues and the initiation of cleavage in egg cells; the primary or critical change in both cases appears to be a temporary and reversible increase in the permeability of the plasma membrane, with accompanying changes in the electrical polarization of the latter.[2] This analogy suggests that the irresponsive condition of the above "over-ripe" starfish eggs is essentially the symptom or expression of an abnormal condition of the plasma membrane. Apparently the latter has in these eggs become *abnormally resistant to changes of permeability;* hence the eggs are irresponsive to the spermatozoön (whose primary action is to increase permeability); hence also they show heightened resistance to cytolytic action—which also depends on increase in surface permeability; this is shown by the slowness with which they undergo the typical post-maturational cytolysis, and also by their increased resistance to pure isotonic sodium chloride solution. If this interpretation is correct, the favorable action of weak ether solution consists essentially in altering the plasma membrane and rendering it more susceptible to the action of permeability-increasing (and hence depolarizing) agencies—*i. e., more irritable,* on the above-mentioned analogy with irritable tissues. Through this means the plasma membrane is restored to an approximately normal condi-

[1] This is very clearly shown in the larvæ of *Arenicola;* cf. the paper just cited, p. 380 ff.

[2] I have discussed the probable basis of this resemblance at some length in an earlier paper in the BIOLOGICAL BULLETIN, 1909, Vol. 17, pp. 20 ff. The title of Loeb's recent book, "Entwicklungserregung des tierischen Eies," also emphasizes this analogy.

tion of responsiveness; the sperm then exhibits its normal action. It is evident that this hypothesis also implies that the other changes in the egg expressive of increased permeability should, after the ether treatment, also follow an approximately normal course. This in in fact the case as regards the post-maturational cytolysis; this change is delayed in the above abnormal eggs, as already described; but it is found to take place in a normal manner in the ether-treated unfertilized eggs. The following descriptions will illustrate both of these effects.

It should first be noted that the degree to which eggs abnormally resistant to fertilization may be rendered normal by the ether treatment is variable. In some of my last summer's experiments the difference between the ether-treated and the untreated eggs of the same lot was slight; in others the contrast was most striking. The degree of resistance to the post-maturational cytolysis is similarly variable. In general it was observed that eggs which showed the most pronounced delay in the onset of this latter change were most readily brought into a normally responsive condition—or "rejuvenated"—by ether. The following series of experiments with three separate lots of starfish eggs—all of which failed with a few exceptions to develop to a blastula stage on simple fertilization without ether treatment—illustrates this variability, as well as the correlation between delay in the post-maturational cytolysis and the possibility of rejuvenation[1] by the ether treatment.

June 30, 1911. Three separate lots of eggs (*A*, *B*, *C*) were used; each lot consisted of the mixed eggs from several starfish. After remaining about one and a half hours in normal sea-water, eggs from each lot were transferred to sea-water containing 0.3 vol. per cent. ether. These eggs were kept in small tightly corked flasks. After 1 hour and 10 minutes in this solution part of the eggs were transferred from each flask to normal sea-water in finger bowls; after washing the eggs free from ether spermatozoa were added. The remainder of the eggs in each flask were similarly transferred to sea-water and fertilized after three and a quarter hours in the ether solution. For each lot there was a *fertilized control* consisting of eggs which had lain untreated in sea-water for about 2 hours and 45 minutes before fertilization.

[1] I use this term because of the analogies it suggests. The eggs are in fact brought by the ether treatment into a condition which is characteristic of eggs produced in the earlier portion of the reproductive cycle. The production of hyper-resistant eggs like the above occurs late in the breeding season, and the phenomenon bears certain analogies to senescence. See below, page 345.

The characteristics and behavior of the eggs from these three lots were respectively as follows:

Lot A.—The great majority of these eggs fail to maturate. A small proportion undergo apparently normal maturation.

Unfertilized Eggs.—22 hours after removal almost all of the mature eggs show the typical opaque and coagulated protoplasm; *i. e.*, post-maturational cytolysis appears normal.

Fertilized Eggs.—Condition *ca.* 20 hours after fertilization.

1. *Untreated (Control) Eggs.*—Many of the immature eggs have typical fertilization membranes; but are otherwise unchanged. The few mature eggs are mostly dead; only one abnormal blastula was found.

2. *Ether-treated Eggs.*—(*a*) Exposed 1 hour 10 minutes. Four or five blastulæ are found in some hundred eggs; little difference from control. (*b*) Exposed 3¼ hours. Little or no improvement over control; a few blastulæ as in (*a*).

Lot B.—Most of these eggs remain immature, but about 20 per cent. undergo apparently normal maturation.

Unfertilized Eggs.—22 hours after removal from the animals most of the mature eggs are opaque and coagulated, but in many the coagulation is less advanced than in normal eggs, and in some the protoplasm remains semi-translucent.

Fertilized Eggs.—Condition *ca.* 20 hours after fertilization.

1. *Untreated (Control) Eggs.*—Almost all of the mature eggs are dead. Many immature eggs have fertilization membranes. Only two abnormal blastulæ are found in several hundred eggs.

2. *Ether-treated Eggs.*—(*a*) Exposed 1 hour 10 minutes. Improvement over the control; a large proportion (about one third) of the mature eggs have formed blastulæ, many of which have begun to gastrulate. (*b*) Exposed 3¼ hours. Also shows a marked improvement over the control, but the larvæ are fewer and less active than in 2*a*.

Lot C.—In this lot of eggs the majority show normal maturation, though a few remain immature.

Unfertilized Eggs.—After 22 hours in sea-water most of the eggs are more or less coagulated, but the degree of opacity is distinctly less than in normal eggs, and a considerable proportion remain translucent—almost like freshly shed eggs.

Fertilized Eggs.—Condition *ca.* 20 hours after fertilization.

1. *Untreated (Control) Eggs.*—Nearly all are dead. Most have membranes and show evidence of having cleaved or fragmented, but many have failed to cleave or even to form membranes. A small proportion of eggs have formed larvæ some of which appear normal: the larvæ though few are more numerous than in the controls of *A* and *B*.

2. *Ether-treated Eggs.*—(*a*) Exposed 1 hour 10 minutes. Striking contrast to control. Almost all of the mature eggs have formed active larvæ, many in the early gastrula stage and swimming at the surface of the water. (*b*) Exposed 3¼ hours. Here also the majority of eggs form larvæ, but these are largely abnormal, and relatively few gastrulæ or surface swimmers are present.

The power of development after fertilization is thus greatly increased after ether-treatment in Lots *B* and *C*, but not in Lot *A*. The mature eggs of Lots *B* and *C* show marked delay in the post-maturational cytolysis; in Lot *C* this delay is greater, and the

action of the ether is correspondingly more favorable, than in Lot *B*. I have already presented evidence that eggs showing this abnormal behavior are characterized by the possession of hyper-resistant plasma membranes. It should be noted that variations in the degree of resistance of this membrane occur regularly in normal eggs. Various facts indicate that the process of maturation is constantly associated with a change in the properties of the plasma membrane. This is shown by the fact that mature eggs undergo cytolysis in $0.55m$ NaCl solution more rapidly than immature eggs;[1] also by the fact that contact with spermatozoa and various forms of artificial treatment cause the separation of the surface-film of mature eggs in the form of a fertilization membrane, a change indicating a superficial cytolytic or permeability-increasing action; while immature eggs are not normally subject to this change.[2] This difference between immature and mature eggs is a constant or physiological feature in the life history of these eggs. The difference between normal eggs and the resistant eggs under consideration is in many respects similar. For some reason the maturation process fails to bring these eggs into the normally sensitive condition in which the permeability of the membrane is readily increased. Hence fertilization is imperfect, even fertilization-membranes failing to form in some cases; in others membranes are formed and cleavage begins, but the latter is characteristically irregular and fails to proceed far. According to this view the failure of development is due not to defective organization of the protoplasm, but simply to the existence of an abnormally resistant plasma membrane. The action of ether consists in restoring the membrane to its normal condition. The response to the spermatozoön then becomes normal.

The experimental evidence in favor of this hypothesis consists at present simply in the fact that such abnormal eggs are rendered normal by ether treatment not only in regard to their response to fertilization, but also in regard to the rate and character of

[1] R. S. Lillie, *American Journal of Physiology*, 1912, Vol. 30, p. ——.

[2] Cf. J. Loeb's experiments on the eggs of *Asterina*, University of California Publications, Physiology, 1905, Vol. 2, p. 150. Spermatozoa and artificial membrane-forming agencies may however produce typical membranes in abnormal immature starfish eggs. Cf. *Journal of Experimental Zoölogy*, 1908, Vol. 5, p. 407.

the spontaneous post-maturational cytolysis; *i. e.*, they undergo complete coagulation within eighteen hours or less, precisely as do normal eggs. Whether the resistance to cytolysis by salt solutions is also decreased I have not yet determined; but the decrease in the resistance to the post-maturational cytolysis—a change supposedly due to the action of certain protoplasmic oxidation products upon the plasma membrane[1]—is clear evidence that the membrane has been brought into a condition more nearly approaching the normal. The experiments to be described show that a return of the normal responsiveness to the spermatozoön is closely correlated with a return of the normal behavior with respect to this spontaneous oxidative cytolysis. In other words, the plasma membranes of the ether-treated "rejuvenated" eggs undergo breakdown in the manner and at the time characteristic of normal eggs.

The following record gives the description of two typical experiments.

July 6, 1911. Eggs were removed at 11:00 A.M. from two lots of starfish, *A* and *B*. In both lots a good proportion of eggs underwent apparently normal maturation. Eggs from each lot were divided into two portions. One portion remained in sea-water; and about four hours after removal from the animals part of these eggs were fertilized; the rest remained unfertilized. The other portion was transferred, two hours after removal, to sea-water containing 0.3 vol. per cent ether; in this solution they remained for one hour and thirty-five minutes; they were then returned to sea-water; to part of these eggs spermatozoa were added, the rest remained unfertilized. The ether-treated and the untreated eggs were fertilized at the same time. The two lots *A* and *B* were treated alike so far as possible. The results of these experiments were as follows:

Lot A. The following was the condition of the eggs *ca.* 22 hours after removal:

1. *Untreated Eggs.* (*a*) *Unfertilized.*—Most mature eggs are coagulated but to a varying degree; some are only slightly darkened, and in a fair proportion the protoplasm remains semi-translucent.

(*b*) *Fertilized.*—All of the mature eggs have formed membranes and most have undergone cleavage or irregular fragmentation; but many remain uncleaved; no blastulæ are present.

2. *Ether-treated Eggs.*—(In 0.3 vol. per cent. ether from 1.00 to 2.35 P.M.)

(*a*) *Unfertilized.*—All mature eggs are completely and uniformly coagulated; there are no partly coagulated or semi-translucent eggs.

(*b*) *Fertilized.*—Decided contrast to 1*b*. Most eggs are dead, but among these there are no uncleaved eggs; numerous blastulæ and gastrulæ are present, many swimming at the surface.

Lot B.—(The condition of the eggs *ca.* 22 hours after removal.)

[1] Since the change is greatly retarded in oxygen-free or cyanide-containing sea-water. Cf. J. Loeb, *Archiv für die gesammte Physiologie*, 1902, *loc. cit.*

1. *Untreated eggs.*—(*a*) *Unfertilized.*—The degree of post-maturation coagulation varies here as in Lot *A*, but a larger proportion of eggs remain semi-transclucent, and many are virtually unchanged in appearance.

(*b*) *Fertilized.*—Most eggs have formed membranes and cleaved or fragmented; a number form membranes but fail to cleave. A few feeble blastulæ are present—a fraction of 1 per cent.; no surface swimmers.

2. *Ether-treated eggs.*—(In 0.3 vol. per cent. ether from 1.00 to 2.35 P.M.)

(*a*) *Unfertilized.*—Marked contraxt to 1*a*. All mature eggs are completely coagulated—opaque, compact looking, without membranes.

(*b*) *Fertilized.*—A large number of active blastulæ and gastrulæ are present, many swimming at the surface. No uncleaved eggs are present, though a good many have died in early stages. More favorable than *A*, 2 (*b*).

The return of the normal responsiveness to fertilization and normal power of development is thus associated with a return of the normal rate of post-maturational cytolysis. This observation was made in six out of nine experiments, at different times and with different lots of eggs, in which ether-treatment led to marked increase in the proportion of eggs undergoing favorable development. In three of the earlier experiments the behavior of the ether-treated unfertilized eggs was not observed; but in all of the six cases where both observations were made concurrently this correlation held. As already mentioned, the degree of improvement effected by the ether has been variable, in correspondence with the degree of abnormality in the eggs. In all of the cases in which the improvement was decided, as in some of those described above, the untreated mature eggs proved largely refractory toward both cytolysis and fertilization; while after the treatment with ether the eggs showed in both respects a behavior approaching the normal. In three other lots of abnormal eggs treatment with ether had no appreciable effect either in accelerating cytolysis or in increasing the proportion of favorably developing eggs; while in one case in which a considerable proportion of untreated eggs developed favorably—about one third forming larvæ—the ether-treated eggs were somewhat *less* favorable than the untreated; in this series the unfertilized eggs, both treated and untreated, showed an apparently normal spontaneous cytolysis. Probably the plasma membranes of these eggs were over-susceptible rather than under-susceptible to increase of permeability. The possible existence of both kinds of abnormalities must be recognized.

Deviation in either direction from the physiological norm would presumably impair the power of development.

General Discussion.

I shall now discuss somewhat more fully the general physiological significance of the above abnormalities and their relations to analogous conditions elsewhere. The above condition, described in general terms, is essentially one of lowered susceptibility to agencies which ordinarily call forth a definite response. Similar conditions exist in other cells and tissues. There are also cases where a tissue is normally irresponsive to certain agencies or conditions, to which however it may be rendered responsive by certain forms of artificial treatment.[1] It seems probable that in all of these cases the condition of the plasma membrane is the essential factor which determines whether the cell or tissue responds to the agency in question or not. This structure, as the most external layer of the cell, is the part most accessible to artificial modification; and if its condition of permeability and electrical polarization plays the controlling rôle in cell-processes which modern investigation tends more and more to indicate, knowledge of the means by which its properties may be altered at will becomes a matter of the highest importance for both the theoretical and the practical aspects of biology.

The abnormalities under consideration appear typically in the eggs of *Asterias forbesii* toward the close of the breeding season. Eggs are abundant at Woods Hole in early June.[2] During the greater part of this month they exhibit as a rule a normal response to fertilization; and if left unfertilized in sea-water at 20° they undergo the above described coagulative

[1] Instances of this are seen in various phenomena of sensitization. A good instance is the hypersensitiveness to contact induced in frog's skeletal muscle by isotonic solutions of sodium citrate, tartrate, sulphate, and certain other salts. Cf. J. Loeb, *American Journal of Physiology*, 1901, Vol. 5, p. 362.

[2] In former years a considerable proportion of starfish collected in August and September have yielded numerous normal eggs. Probably these starfish were of a different species from the above—presumably *A. vulgaris*. During the last few years this form seems to have become rare in the neighborhood of Woods Hole, and eggs have been difficult to obtain later than June. Two species of *Asterias*, *forbesii* and *vulgaris*, are recognized as occurring in this region; cf. H. L. Clark, Bulletin of the U. S. Fish Commission, 1902, p. 552.

cytolysis within 12 to 15 hours or less. Toward the end of June eggs become fewer and more variable in quality, many fail to maturate and the mature eggs on fertilization tend to cleave irregularly and largely die before reaching the blastula stage; a large proportion of eggs show the abnormalities described above; increased resistance to fertilization and to cytolytic alteration is especially characteristic and indicates that the plasma membrane has become abnormally resistant to changes in permeability.

I have described the peculiarities of these eggs in sufficient detail above, and have already briefly discussed the physiological nature of the abnormalities. The failure to respond normally to fertilization becomes intelligible on the hypothesis that the essential or critical event in the initiation of cell-division is a temporary and reversible increase in the ionic permeability of the plasma membrane. Such a change involves a decrease in the electrical polarization of the membrane, and it appears probable—as in the analogous case of muscle and nerve—that this change of polarization, and not the mere increase of permeability as such, is the critical event which initiates the rhythmical series of physical and chemical processes of which cleavage is the normal expression.[1] In order that the egg may respond to the contact and entrance of the spermatozoön in a normal manner, its plasma membrane must have a certain definite physico-chemical constitution such that the substances transmitted by the spermatozoön[2] may effect an increase of permeability which in rate and degree approximates a certain norm. This implies a certain degree of resistance to change of permeability; if this resistance is abnormally great, the response fails to occur or is imperfect; if abnormally low, the spermatozoön effects too great and too lasting an increase in permeability

[1] The astral radiations of mitosis are probably the expression of potential differences between different regions of the cell, arising in consequence of altered polarization of the limiting membranes. The alternate appearance and disappearance of the radiations indicates a rhythm of changing polarization of the limiting membranes. For a further discussion cf. my papers in BIOLOGICAL BULLETIN, 1909, *loc. cit.*; *Amer. Jour. Physiol.*, 1910, Vol. 26, pp. 128 ff.; *Journal of Morphology*, 1911, Vol. 22, p. 711.

[2] According to Loeb, certain lysin-like bodies (cf. "Entwicklungserregung," p. 247). I have suggested calling these "membranolysins" to indicate the essential nature of their action.

resulting in early death or cytolysis—just as occurs in eggs subjected to a simple membrane-forming treatment without subsequent exposure to hypertonic sea-water.[1] Eggs which begin cleavage, but fragment and break down before proceeding far in development, possibly belong to this latter class. If normal development is to follow fertilization, the properties of the plasma membrane cannot, on the present hypothesis, depart widely from a constant mean or physiological norm.

The failure of the above eggs to respond normally to fertilization, as also their resistance to cytolysis, is thus to be regarded as the expression of a highly resistant condition of the plasma membrane. The latter fails readily to undergo the increase of permeability essential to these changes,—probably because of abnormalities in the nature, state, or proportions of its chemical constituents. The essential effect of the treatment with ether is to restore the normal properties of the membrane. There is, however, no reason to believe that this effect is specific to ether. In some of my last summer's experiments a similar though less favorable effect was produced by exposure to isotonic sodium chloride solution and—in one case—to a 0.1 per cent. solution of chloral hydrate in sea-water. In its general form the problem relates to the essential nature of the modification which these substances induce in the egg, and by which the latter is brought from an irresponsive condition into one in which it shows a normal response. Light is thrown on this problem by the conditions in irritable tissues such as muscle and nerve.

A close analogy exists between the initiation of cell-division in eggs or other resting cells, and the response of an irritable tissue to stimulation. In both cases the initial or critical event is apparently a temporary increase in surface-permeability, with accompanying changes in the electrical polarization of the limiting membranes. The means by which refractory eggs may

[1] The second part of the treatment appears to effect a return of the permeability—which has been increased by the membrane-forming treatment—to the normal (cf. *Amer. Jour. Physiol.*, 1911, Vol. 27, p. 289). Godlewski (*Archiv für Entwicklungsmechanik*, 1911, Vol. 33, p. 225) has independently reached a similar conclusion with regard to the essential nature of the effect produced by the hypertonic sea-water.

be rendered normally responsive is thus analogous to that by which the responsiveness of muscle and nerve to stimulation may be increased. An irresponsive condition in living muscle and nerve may be due to anæsthesia, fatigue, electrotonus, toxic action, or other changes of state. The stimulating action of small doses of alcohol[1] and other narcotics during fatigue suggests an analogy which is probably not without significance. It is known that traces of various lipoid-solvent substances very generally increase irritability, or the rate of spontaneous activity, in the most various cells and tissues (leucocytes, cilia, the heart, etc.).[2] The increased responsiveness of the above eggs after ether treatment is a phenomenon of the same general kind. It would appear that the condition of the lipoids in cells determines the readiness of response or the rate of spontaneous activity; and that slight impregnation of the lipoids in the membrane with a lipoid-solvent facilitates in this structure the alteration which conditions the permeability-increase and polarization-change of stimulation.

Certain salts markedly increase the irritability of muscle and nerve,—*i. e.*, induce sensitization.[3] Treatment with salt solutions may also restore fatigued muscles to an irritable condition. Frog's skeletal muscles immersed in isotonic sodium chloride solution and made to contract by successive electrical stimuli until irresponsive promptly recover irritability if immersed in isotonic sodium bromide, nitrate or iodide solutions. Sodium iodide restores irritability to muscles which have been fatigued in sodium bromide or nitrate solutions, but chloride has no such action; *i. e.*, the order of the salts cannot be reversed. The restorative effect is rapid, and evidently depends on a surface action, the colloids of the membrane being apparently brought into a condition favorable for stimulation—apparently a condition of increased dispersion.[4] As already described, sodium chloride solution may produce an analogous increase of respon-

[1] The action of small quantities of alcohol in counteracting fatigue in excised frog muscles has been well shown by Lee and Salant, *Amer. Jour. Physiol.*, 1902, Vol. 8, p. 61.

[2] Cf. footnote 2, page 331.

[3] Cf. footnote 1, page 338.

[4] C. Schwarz, *Archiv für die gesammte Physiologie*, 1907, Vol. 117, p. 161.

siveness in refractory starfish eggs. Schwarz's observations, as well as my own with abnormal starfish eggs, thus belong to that general class of cases in which the responsiveness of cells is increased by treatment with salts or low concentrations of lipoid-solvents. The response of voluntary muscle to various forms of chemical stimulation may be increased by brief immersion in isotonic solutions of various sodium salts; in the case of salts which do not precipitate calcium, this sensitizing action increases with variation in the nature of the anion in the following general order: $Cl < Br < NO_3 < ClO_3 < CNS$ and I, an order corresponding to the order of increasing effectiveness in promoting colloidal dispersion.[1] Interpreted in terms of the membrane theory, these facts mean that the readiness with which the plasma membrane undergoes increase in permeability may be increased either by altering the general state of the colloids in the membrane, or by slightly altering that of the lipoids alone.

We conclude that the effect produced by salts and weak ether solutions in increasing the responsiveness of refractory eggs to fertilization is comparable with the sensitization of irritable tissues by these substances; also that in both cases the essential change consists in an increase in the readiness with which the plasma membrane undergoes the critical change of permeability and of electrical polarization.

It is to be noted that the resistance of eggs to fertilization by foreign sperm may also be decreased by chemical treatment, as Loeb discovered several years ago.[2] Heightening the alkalinity of the medium has this effect. This characteristic and striking effect is probably an expression of a very general action of weak alkali. Many facts indicate that slight increase in the alkalinity of the medium usually increases the readiness with which the permeability of cells is altered: cell-division is accelerated, the irritability of irritable tissues and the rate of activity of automatic tissues is increased, the cytolytic action of salt solutions is accelerated, and in unfertilized eggs membrane formation and the initiation of cleavage may be induced.[3] It remains to be determined whether

[1] R. S. Lillie, *Proceedings of the Society for Experimental Biology and Medicine*, New York, 1910, Vol. 7, p. 170.

[2] J. Loeb, University of California Publications, Physiology, 1903, Vol. 1, p. 1.

[3] Cf. J. Loeb, *Archiv für die gesammte Physiologie*, 1907, Vol. 118, p. 7.

alkali as well as ether can overcome the resistance of over-ripe starfish eggs to fertilization by their own sperm; also whether treatment with weak solutions of ether or other lipoid-modifying substances, as well as with weak alkali, can render cross-fertilization possible. The case of the eggs of hermaphrodite animals, which are irresponsive to sperm from the same individual but not to that of other individuals, may possibly belong in part to the present category. Morgan[1] found that the eggs of *Ciona*, which furnish a typical instance of this behavior, could be fertilized by spermatozoa from the same individual in weak solutions of ether, ammonia or alcohol; but he is inclined to attribute the effect to the stimulating action of these substances on the sperm, rather than to an alteration of the egg. In one experiment, however (p. 147), in which the spermatozoa alone were treated with ether before adding to the egg, few eggs were fertilized, while when both eggs and sperm were so treated the percentage of fertilization was high (in one case 85 per cent. as compared with 4 per cent.). This experiment suggests that the ether produces its effect not merely by increasing the motility of the sperm, but also by altering the condition of the egg, as in the case of *Asterias*. Another interesting case in which the egg is rendered refractory to fertilization has recently been described by Godlewski.[2] If the spermatozoa of *Chætopterus* and of *Sphærechinus* are mixed, both are found after a few minutes to have completely lost the power of fertilizing the eggs of *Sphærechinus*.[3] Eggs left exposed to this

[1] Morgan, *Journal of Experimental Zoölogy*, 1904, Vol. 1, p. 135.

[2] Godlewski, *Archiv für Entwicklungsmechanik*, 1911, Vol. 33, p. 233.

[3] The spermatozoa in this mixture retain their motility in spite of their having ost the power of affecting the egg (*loc. cit.*, p. 240). It would be interesting to know if the motility undergoes *any* decrease. Apparently the surface layer of the spermatozoön is modified, since there is no transmission of membranolysins to the egg. This would imply heightened resistance to permeability-change in the plasma membrane of the spermatozoön; and such a change would presumably involve decreased motile activity—*i. e.*, a slower rhythm in the variation of ionic permeability and polarization conditioning the movements. Various facts indicate that in normal fertilization the spermatozoön and the egg affect each other *mutually* in a somewhat similar manner, and that a cytolytic or permeability-increasing action is exercised by the egg upon the sperm as well as by sperm upon the egg. Thus the sperm usually ceases its movements soon after contact with the egg, and often only a portion enters the latter; a cytolytic or disintegrative change in the spermatozoön is thus indicated. The plasma membrane of the sperm might—as apparently in Godlewski's experiments—be rendered sufficiently hyper-resistant to prevent this mutual cytolytic action without entire loss of motility.

sperm mixture are at first unaffected, but in the course of half an hour they become so modified that fertilization with normal *Sphærechinus* sperm is impossible. The egg is thus deprived of the power of response to its own spermatozoa.[1] Whether this condition of irresponsiveness (which is comparable to paralysis) may be removed by artificial treatment such as the above has apparently not been determined; but from the analogies with the conditions just described there is every reason to believe that this would readily be found possible.[2]

In conclusion I wish briefly to indicate the bearing of the above observations on the general theory of pathological alterations in cells. The conclusion that many pathological conditions have their primary origin in abnormalities of the limiting membranes of cells is an obvious corollary of any view that regards such membranes—which are essentially insulating surface-films of varying ionic permeability and electrical polarization—as largely controlling the rate and character of the cell-processes. If stimulation depends primarily on altered polarization of the plasma membrane due to increased ionic permeability, it is clear that a normal response, in the case of any cell, implies a definite condition of the membrane. If this condition is permanently altered the cell processes inevitably undergo derangement, and pathological changes follow. Such a deranged condition, if not too far advanced, may be rectified by restoring the membrane to its normal condition. How this may be accomplished is illustrated by the case of the abnormal starfish eggs described above. It is clear from the cytolytic effects produced by many toxines that they cause abnormal increase in the permeability of the membranes; and in all probability their destructive action is in many cases directly due to this surface action. The alteration caused by a toxic agent may consist primarily either in increasing or in decreasing the permeability normal to the membrane, or in altering in either direction the readiness with which the latter undergoes change. Evidently the plasma membrane, as an

[1] A similar and reversible effect may be produced by treating the egg with certain salt solutions, as Mathews and Newman showed some years ago for *Fundulus* eggs; cf. BIOLOGICAL BULLETIN, 1905, Vol. 9, p. 378.

[2] Evidence that this change is in fact reversible is seen in the effects of washing the eggs thoroughly in sea-water after the treatment with the sperm mixture. A partial return of responsiveness to the spermatozoön was observed (p. 236).

insulating—and hence semi-permeable—layer on the integrity of which the normal composition of the living substance depends, cannot undergo marked and prolonged increase of permeability without alteration in the nature and proportion of the cell-constituents; this involves altered chemical organization and eventual derangement of the cell-processes.[1] In most of the abnormal conditions considered above the membrane appears to have undergone the opposite kind of modification, becoming abnormally impermeable and resistant to changes of permeability. Such a condition is essentially one of irresponsiveness, and is in a sense pathological, although it differs from a condition of permanently increased permeability in involving no loss of material from the cell; hence the possibility of restoring the normal properties of the cell—by bringing the permeability again to the normal—should theoretically be greater in this class of cases than in the other.[2]

Perhaps the condition of the above resistant starfish eggs is not properly to be called pathological, since the change in the properties of the eggs toward the close of the breeding season is presumably a constant one, and hence normal in a physiological sense. The eggs merely become hyperresistant to fertilization; *i. e.*, with increasing age the metabolism of the ovaries undergoes alteration so as to lead to the production of eggs having more resistant membranes than before. The cycle of egg-production shortly afterwards comes to a close. The phenomenon bears in certain respects a marked resemblance to senescence, and its conditions may throw light on the physiology of the latter process. In old age the irritable tissues become less and less responsive, and the rate of metabolism is correspondingly lowered. Irre-

[1] I have dwelt on these considerations at somewhat greater length in my earlier paper in this journal, 1909, Vol. 17, p. 197 et seq. The fundamental importance of the part which alterations of the membranes play in pathological processes was first recognized by Zangger. The following quotation will illustrate: "Die normale typische Permeabilität der Membranen ist also Voraussetzung der normalen Lebensfunktionen. Dauernd veränderte Permeabilität der Membranen bedeutet Pathologie, pathologischen Stoffwechsel," etc.: Vierteljahrsschrift d. Naturf. Ges. Zürich, 1906, Vol. 51, p. 432. Cf. also *ibid.*, 1907, 1908, and the other papers of Zangger and his students, especially Frei and Stoffel, for a fuller development of these ideas, together with experimental data bearing on the relations of membrane changes to pathological processes, immunity, and related phenomena.

[2] For further discussion of this subject cf. my earlier paper in *American Jour. Physiol.*, 1910, Vol. 26, pp. 112 et seq.

sponsiveness, as already pointed out, implies a state of the plasma membrane in which changes of permeability and of electrical polarization are produced with difficulty. The increasingly resistant character of the membranes implies altered composition. Possibly the condition in over-ripe eggs, as well as in senescence, results from a progressive accumulation or adsorption at the phase-boundaries, *i. e.*, in the membranes, of materials which for some reason are not readily eliminated by the organism.[1] A similar view has recently been expressed by Child.[2] According to his theory "senescence in nature consists physiologically in a decrease in the rate of metabolism, and this is determined morphologically by the accumulation in the cell of structural obstacles to metabolism, *e. g.*, decrease in permeability, increase in density, accumulation of relatively inactive substances, etc." Minot's[3] theory that senescence is the expression of a progressively increasing differentiation of cells, *i. e.*, of an increase in the proportion of separated solid structural material, bears a certain resemblance to this view. The view which I have suggested above is distinctive only in so far as it attributes the essential change to a modification of the *membranes*. It is, theoretically at least, within the possibilities of physiological science to prevent or retard this accumulation of inert materials in the membranes and so to delay senescence. Or the already modified membranes might, if not too profoundly altered, be restored to a normal condition by certain forms of treatment. The simpler the metabolism and the less widely differentiated the tissues, the greater would appear to be the possibility of such "rejuvenescence" of the organism as a whole. In one large group, the Protozoa senescence seems not to be an inevitable occurrence; the earlier interpretation of conjugation as a process whose essential rôle is to counteract an innate tendency to senescence has been discredited by the work of Calkins and Woodruff. The conditions in Metazoa differ from those in Protozoa chiefly in their greater complexity, but probably in no other essential respect.

[1] Probably certain colloidal (*i. e.*, indiffusible) and chemically refractory by-products of metabolism.

[2] Cf. Child, *Archiv für Entwicklungsmechanik*, 1911, Vol. 31, p. 537.

[3] C. S. Minot, "The Problem of Age, Growth and Death." New York and London, 1908.